Upsala, January 27. 1935.

MH 76 F4

Dear Professor Heidelberger.

First I wish to thank you for your letters of November 30th and December 13th.

I have not yet got the sample of lipoid-free thyroglobulin from Dr.Cavett, so I wonder if he has had a sample ready.

I have discussed your proposal of dividing the work over two articles with professor Svedberg, but he thought it just as good to keep the whole inside one article, but he proposed to change the cronological order of the different divisions in the way I have done now in the MS; he found it more natural to have the isoelectric point section before the centrifuge work.

We found it necessary to extend the section about the sedimentation constant somewhat, and I found it necessary to recalculate the run no: 20, because it was quite obvious that there were two components present, which could be calculated separately.

Professor Svedberg has added the small note just before "summary" about the grants, because the whole expenses connected with the centrifuges are paid from these grants.

The value for the molecular weight I gave you in my letter of November 19th is not correct, the correct value is about 650-700 000, but you only get this value, when the concentration is higher than ½ %; below this concentration there is a rather strong dissociation taking

place. We did not find this error (in the measurements), before we got the values calculated for a second wavelength, then the values for the concentration gradient in the cell would not at all correspond, and we had to control all the old measurements, and then we found that there was a very big error in one of the fundamental measurements (0,5 mm, that is equal to a whole turn on the dial on the measuring microscope) in 4 of the calculated 6 equilibrium runs. These measurements were made by a new girl we had to assist miss Norling, but we had her only for a short time, because she was quite impossible for these measurements. When we then got the equilibrium runs recalculated, they showed a very strong drift in the values for the lower concentrations as you see in the equilibrium run tables, but at the concentrations from about ½ % and upwards the calculated molecular weight seems to be rather constant; yet the thyroglobulin is always containing some higher molecular components. Under these conditions (strong and changing dissociation and more molecular species) it is not allowed (as I told you here in Upsala) to use the expression we first used for the preliminary calculation of equilibrium runs no.1, but the method I have derived for the calculation of the molecular weight is also not so sure in this case as it will usually be, because one has to extrapolate the concentration curve quite a bit due to the impossibility (caused by the high molecular component) of measuring the plate quite down to the bottom of the cell. This means that the error in these equilibrium runs could perhaps be some 5–10 %, but the values given are probably rather too low than too high. In case of the dilute solution these equilibrium runs are rather uncertain, so that they cannot give any informations about the size and amount of the dissociation products. The sedimentation velocity runs tell us too that there is a strong dissociation taking place, when the

concentrations decrease below ½ %. This dissociation is causing the strongly sloping baseline you generally found in the velocity runs, where you in nearly all the experiments used a concentration much lower than ½ % (about 0,17 %); in the single run, no.17, with a higher concentration the sloping baseline has disappeared. I propose that we introduce two more figures (no.3 and 4) showing the photometer curves of the two runs no. 17 and 14. You would perhaps be so kind as to get these and the electrophorese figure drawn like the fig.2, so that they may be all alike.

This strong dissociation by dilution is perhaps of some physiological interest, but I have not introduced anything about this in the MS, because I think it much better, if you yourself make the necessary change in "Discussion". The small alterations made in this part are made by Professor Svedberg. You see, it is very easy to get quite a number of different hemocyanins with both higher and lower molecular weights.

In "Discussion" I think that the part where you speak about the old preparations should perhaps be changed somewhat; it is correct that the sedimentation constant of the main component is the same, but this main component is just in old preparations a much smaller part of the total protein present, especially in the case of the human thyroglobulin, which indicates that a change in the protein (taken as a whole) is taking place. It would perhaps be possible just by a new precipitation of the thyroglobulin to get a new preparation quite as homogeneous as the freshly prepared thyroglobulin.

I wonder if one can say that thyroglobulin has an unusually low isoelectric point. Tiselius has found the isoelectric point for horse serum albumin at pH 4,88, by later determinations I have always found nearly the same or perhaps a little lower value. The horse serum globu-

lin has an isoelectric point of about pH 5,5, but we know nothing about the isoelectric point for the hog albumin and globulin, and they may be lower than those of the horse; I have very often thought of making the isoelectric point determinations on quite a lot of serum albumins and globulins from different species, but so far I have not got the time to do so.

I really think that this work on the thyroglobulin has been very interesting and that it ought to be continued on different parts, but I am afraid that it would be too long now to start a discussion with you about that, but perhaps some of your assistants are later on coming to Upsala?

I have forgotten to ask Professor Svedberg whether he has made any arrangements with you as regard to the reprints; generally they want to get (or buy) 200 exp. for the institute.

Would you please be so kind to correct at least the english in the places in the MS I have written? I regret very much that we can't have the possibility of discussing the whole MS together personally, that would have been very interesting.

At last I wish to beg you to excuse me for having been so late with the MS.

With my kindest regards to you and Mrs.Heidelberger,

Sincerely

Kai O. Pedersen.